DATA EVALUATION RECORD

DIMETHOATE

Study Type: Repeated Dose (28-Day) Oral Toxicity Study in Rats

Work Assignment No. 1-01-38 (MRID 46288001)

Prepared for
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Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Repeated Dose (28-Day) Oral Toxicity [diet] - rats; OPPTS 870.3050; OECD 407.

PC CODE: 035001 **DP BARCODE:** 305930
TXR#: 0052750 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Dimethoate (Batch # 20522-00; 99.1% a.i.)

SYNONYMS: BAS 152 I; *O, O*-dimethyl *S*-(*N*-methylcarbamoyl-methyl)-phosphorodithioate

CITATION: Kaspers, U., Kaufmann, W., Deckardt, K., *et al.* (2004) Dimethoate: range-finding study in Wistar rats; administration via the diet over 4 weeks. Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany. Laboratory Project Id.: 30S0466/99143, April 20, 2004. MRID 46288001. Unpublished

SPONSOR: Dimethoate Task Force, Mannheim, Germany

EXECUTIVE SUMMARY: In a repeated dose oral toxicity study (MRID 46288001), Dimethoate (Batch # 20522-00; 99.1% a.i.) was administered continuously in the diet to Wistar (CrlGlxBrlHan:WI) rats (5 animals/sex/dose) at nominal dose levels of 0, 1.0, 3.0, or 12.5 mg/kg bw/day (actual doses 0/0, 0.83/0.85, 2.48/2.60, and 10.38/11.00 mg/kg bw/day males/females) for up to 28 days.

No effects of treatment were observed on clinical signs, mortality, food consumption, water consumption, hematology, clinical chemistry, organ weight, or gross pathology.

Inhibition of either erythrocyte or serum cholinesterase (ChE) activity was seen on day 2 of examination period. On Day 8 in the 12.5 mg/kg/day females only, erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 34%. On Day 29 in the 12.5 mg/kg/day males, decreases ($p \leq 0.05$-0.01) were observed in serum cholinesterase (↓23%), erythrocyte cholinesterase (↓70%), and brain cholinesterase (↓65%) activities. On Day 29 in the females, decreases ($p \leq 0.01$) were observed at 12.5 mg/kg/day in serum cholinesterase (↓38%), at ≥3.0 mg/kg/day in erythrocyte cholinesterase (↓26-70%), and at 12.5 mg/kg/day in brain cholinesterase (↓70%)

activities.

The LOAEL for serum and brain cholinesterase inhibition was 12.5 mg/kg/day. The NOAEL was 3.0 mg/kg/day.

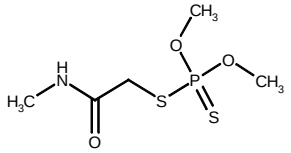
The LOAEL for erythrocyte cholinesterase inhibition was 3.0 mg/kg/day. The NOAEL was 1.0 mg/kg/day.

This 28-day repeated dose oral toxicity study is classified as **acceptable/guideline** and satisfies the Guideline requirements (OPPTS 870.3050; OECD 407) for a 28-day oral toxicity study in rats.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material:		Dimethoate
	Description:	White solid
	Batch #:	20522-00
	Purity:	99.1% a.i.
	Compound Stability:	Stable in the diet for up to 8 days at room temperature or at freezer conditions
	CAS # of TGAI:	60-51-5
	Structure:	

2. Vehicle: diet

3. Test animals			
	Species:	Rat	
	Strain:	Wistar (CrlGlxBrlHan:WI)	
	Age at study initiation:	49±1 days	
	Mean weight range on Study Day 0:	190.0-196.5 g males; 139.3-149.1 g females	
	Source:	Charles River Germany GmbH, Sulzfeld, Germany	
	Housing:	Individually in stainless steel (type DK III) wire mesh cages.	
	Diet:	Ground Kliba maintenance diet # 3433 (Provimi Kliba, SA, Kaiseraugst, Switzerland), *ad libitum*	
	Water:	Tap water *ad libitum*	
	Environmental conditions:	**Temperature** **Humidity** **Air changes** **Light cycle**	20-24°C 30-70% Not reported 12 hrs light/12 hrs dark
	Acclimation period:	Approximately 14 days	

B. STUDY DESIGN

1. In life dates: Start: January 29, 2002 End: February 27, 2002

2. Animal assignment: Animals were randomly assigned (stratified by body weight) to the test groups noted in Table 1.

Table 1. Study design[a]

Test Group	Nominal Dose (mg/kg bw/day)	Actual Dose (mg/kg bw/day)	# Male	# Female
Control	0	0/0	5	5
Low	1.0	0.83/0.85	5	5
Mid	3.0	2.48/2.60	5	5
High	12.5	10.38/11.00	5	5

a Data were obtained from pages 18 and 56-57 of the study report.

3. Dose selection rationale: It was stated that the doses used were suggested by the Sponsor, with 1 mg/kg/day expected to be the NOAEL for the study. No other information was provided.

4. Treatment preparation, administration, and analysis: Dosing formulations were prepared weekly; however, storage conditions were not provided. The test compound was weighed, dissolved in acetone, and mixed with a small amount of feed. This premix was then diluted with an appropriate amount of feed to obtain the desired dietary concentration. Homogeneity (top, middle, and bottom) was verified in the low and high concentration dose formulations, and concentration was verified on all dose levels from samples from the first formulation. Prior to the study, stability was verified in a 1 ppm dietary formulation, using either acetone or water as a carrier, and following storage for up to 8 days at either ambient temperature or freezer temperature (not specified).

Results

Homogeneity (% coefficient of variation)
6.0 mg/kg = 7.9%
73.0 mg/kg = 3.5%

Stability (range of % of initial concentration)
stored 8 days at room temperature: 93.4-94.1%
stored 8 days at freezer temperature: 104.0-115.1%

Concentration (range of % of nominal concentration)
6.0 mg/kg = 81.4-95.3%
18.0 mg/kg = 88.4-95.8%
73.0 mg/kg = 86.2-92.3%

It was noted that the samples were stored for several months at freezer temperatures prior to analysis, and this could account for the low concentration values. The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the study animals was acceptable.

5. Statistics: Data were subjected to the following statistical procedures:

Parameter	Statistical test
Food consumption, body weight and body weight gains, and food efficiency	Two-sided Dunnett's test
Hematology, clinical chemistry, cholinesterase activity, and organ weights	Two-sided Kruskal-Wallis test followed by two-sided Wilcoxon's test if significance found

Significance was denoted at $p \leq 0.05$ and $p \leq 0.01$. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. Cageside Observations: Animals were inspected for signs of toxicity and mortality twice daily on weekdays and once daily on weekends and holidays.

b. Clinical Examinations: Detailed clinical examinations were conducted daily.

c. Neurological Evaluations: Neurological evaluations were not performed.

2. Body weight: Animals were weighed prior to the beginning of the study, on Day 0, and then weekly thereafter. Body weight gains were calculated for Days 0-7, 0-14, 0-21, and 0-28.

3. Food and water consumption and compound intake: Food consumption for each animal was determined weekly and calculated as g/animal/day. Food efficiency (group means) was calculated based on individual values for body weight and food consumption at weekly intervals. Water consumption was observed daily by visual inspection of the water bottles; data were not reported. Mean daily compound intake was calculated based on the individual body weights, food consumption, and weekly adjusted concentrations of test substance in the diet.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were not performed and are not a guideline requirement (OPPTS 870.3050; OECD 407) for a 28-day oral toxicity study.

5. Hematology & clinical chemistry: Blood was collected from the retroorbital venous plexus of fasted animals (5/sex/dose) for hematology and clinical chemistry from all animals prior to the start of the study, and on Days 2, 8, and 29. Summary tables were presented for Day 29 only. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
X	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
X	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

6. Urinalysis: Urinalysis was not performed and is not a guideline requirement (OPPTS 870.3050; OECD 407) for a 28-day oral toxicity study.

7. Cholinesterase activity: Cholinesterase activity was determined using a modification of the method of Ellman, with DTNB (serum and brain) or DTNA (erythrocyte) as the color reagents. Serum and erythrocyte cholinesterase activity were measured in all animals prior to the start of the study, and on Days 2, 8, and 29. Brain cholinesterase activity was measured in all animals in samples obtained at study termination.

8. Sacrifice and pathology: All animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*+
	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*	XX	Epididymides*+		Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*		Skeletal muscle
	Pancreas*	X	Seminal vesicles*		Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*		Mammary gland*		
	Nose*	X	Vagina		
	Pharynx*				
	Larynx*				

* Recommended for 28-day oral rodent studies based on Guideline 870.3050
\+ Organ weights required for rodent studies.

All tissues were fixed in 4% formaldehyde solution. Parts of the liver dedicated for routine investigation were fixed in Carnoy's solution and embedded in paraplast. Microscopic pathology was not performed.

II. RESULTS

A. OBSERVATIONS

1. Clinical signs of toxicity: No clinical signs of toxicity were observed.

2. Mortality: All animals survived to study termination.

3. Neurological evaluations: Neurological evaluations were not performed.

B. BODY WEIGHT AND BODY WEIGHT GAIN: Body weights and body weight gains in

the males are presented in Table 2. In the 12.5 mg/kg/day males, body weights were slightly decreased (not significant [NS]) on Days 21 and 28 (↓2-3%). Cumulative body weight gains decreased (NS) throughout the study such that overall (Days 0-28) body weight gains were decreased (NS) by 12%. The changes in body weight gain in high dose group was slight and not statistically significant. Such a small change was equivocal considering the absolute body weights were comparable among the treated and the control animals. In treated females, no changes in both body weights or body weight gains were seen.

Table 2. Body weights and cumulative body weight gains (g) in male rats dosed with Dimethoate for up to 28 days.[a]

Observations	Dose (mg/kg/day)			
	0	**1.0**	**3.0**	**12.5**
Body Weight				
Day 0	193.4±11.2	193.0±6.4	190.0±10.6	196.5±7.5
Day 7	231.0±16.4	227.1±6.6	225.0±12.8	233.6±9.4
Day 14	257.7±17.2	253.5±3.8	249.5±17.6	257.0±11.1
Day 21	284.2±18.7	279.9±6.3	276.0±23.5	280.0±12.4 (↓2)
Day 28	305.1±20.1	301.5±7.9	295.6±24.3	294.6±13.4 (↓3)

Body Weight Gain				
Days 0-7	37.6±5.8	34.1±5.2	34.9±3.8	37.2±4.2 (↓1)
Days 0-14	64.3±8.7	60.5±4.5	59.4±10.5	60.5±5.9 (↓6)
Days 0-21	90.8±10.7	86.9±4.3	85.9±15.0	83.5±7.9 (↓8)
Days 0-28	111.7±13.9	108.5±6.1	105.5±15.1	98.2±9.6 (↓12)

a Data were obtained from pages 50 and 52 of the study report.

C. FOOD AND WATER CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: No effect of treatment was noted on food consumption.

2. Food efficiency: Food efficiency was decreased ($p \leq 0.05$) in the 12.5 mg/kg/day males on Day 28 (↓28%). No other treatment-related effects were observed on food efficiency.

3. Water consumption: It was stated that no changes were observed on water consumption; however, summary data were not provided.

4. Compound consumption: Actual test substance intakes, based on food consumption, body weights, and dietary analyses, are presented in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: Ophthalmoscopic examinations were not performed.

E. BLOOD ANALYSES

1. Hematology: No treatment-related effects were observed on hematology. Mean corpuscular hemoglobin concentration was slightly increased ($p \leq 0.05$) in the 12.5 mg/kg/day females on Day 29 (↑3%), but this finding was considered incidental. No other differences ($p \leq 0.05$) were observed.

2. Clinical Chemistry: No effects of treatment were noted on clinical chemistry. On Day 29, phosphate was increased ($p \leq 0.05$) in the 12.5 mg/kg/day males (↑17%), and urea was increased ($p \leq 0.05$) in the 1.0 and 12.5 mg/kg/day females (↑21-26%). However, these findings were minor and/or not dose-related, and were considered incidental. No other differences ($p \leq 0.05$) were observed.

F. URINALYSIS: Urinalysis was not performed.

G. CHOLINESTERASE ACTIVITY: Cholinesterase activity (ChE) data are presented in Table 3. An increase ($p \leq 0.05$) was observed in erythrocyte cholinesterase activity in the 1.0 mg/kg/day females (↑9%) on Day 2, but this finding is considered to be incidental. Inhibition of either erythrocyte or serum ChE activity was seen on day 2 of examination period. On Day 8 in the 12.5 mg/kg/day females only, erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 34%.

On Day 29 in the 12.5 mg/kg/day males, decreases ($p \leq 0.05$-0.01) were observed in serum cholinesterase (↓23%), erythrocyte cholinesterase (↓70%), and brain cholinesterase (↓65%) activities. On Day 29 in the females, decreases ($p \leq 0.01$) were observed at 12.5 mg/kg/day in serum cholinesterase (↓38%), at ≥3.0 mg/kg/day in erythrocyte cholinesterase (↓26-70%), and at 12.5 mg/kg/day in brain cholinesterase (↓70%) activities.

Table 3. Cholinesterase activities (μkat/L) in rats dosed with Dimethoate for up to 28 days.[a]

Measurement	Dose Group (mg/kg/day)			
	0	1.0	3.0	12.5
Males				
Serum (Day -7)	13.88±1.97	13.54±2.32	12.78±1.64	15.05±2.35
Serum (Day 2)	12.98±1.89	12.98±2.61	11.71±1.06	13.53±2.69
Serum (Day 8)	12.44±2.14	12.57±2.58	11.28±1.37	11.70±2.10
Serum (Day 29)	11.01±1.51	11.54±2.01	10.14±1.21	8.49±1.44* (↓23)
Erythrocyte (Day -7)	32.26±7.71	34.17±7.24	32.82±7.74	33.91±1.64
Erythrocyte (Day 2)	37.21±3.03	36.61±2.18	36.95±3.80	34.71±3.29
Erythrocyte (Day 8)	34.16±4.83	33.06±4.29	34.44±4.34	24.90±4.44
Erythrocyte (Day 29)	38.69±3.53	35.07±4.08	33.90±3.74	11.77±3.66** (↓70)
Brain (Day 29)	2.24±0.69	2.27±0.66	1.78±0.52	0.78±0.38** (↓65)
Females				
Serum (Day -7)	25.15±4.06	24.53±1.53	28.06±6.09	26.48±5.29
Serum (Day 2)	29.46±8.18	24.81±2.91	33.29±8.53	29.60±6.95
Serum (Day 8)	33.74±3.85	28.66±2.50	38.99±8.68	27.51±4.69
Serum (Day 29)	46.53±5.85	40.12±6.11	47.65±10.13	28.62±4.94** (↓38)
Erythrocyte (Day -7)	35.88±2.65	38.97±4.06	35.23±5.76	39.72±4.21
Erythrocyte (Day 2)	37.18±0.55	40.40±1.02* (9)	36.38±4.61	35.23±2.17
Erythrocyte (Day 8)	32.31±2.05	36.15±3.67	31.44±4.08	21.20±2.01** (↓34)
Erythrocyte (Day 29)	35.86±1.23	36.83±2.11	26.70±4.43** (↓26)	10.88±1.74** (↓70)
Brain (Day 29)	2.18±0.37	3.11±0.46** (↑43)	1.53±0.57	0.65±0.11** (↓70)

a Data were obtained from pages 70-75 of the study report. Percent differences from controls (calculated by reviewers) are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$

H. SACRIFICE AND PATHOLOGY

1. Organ weight: No treatment-related effects were observed on organ weights. In the females, absolute and relative (to body) ovary weights were increased ($p \leq 0.05$) in the 1.0 (↑30-35%) and 12.5 (↑25-27%) mg/kg/day groups; relative kidney weights were decreased ($p \leq 0.01$) in the 1.0 mg/kg/day group (↓10%); and relative brain weights were increased ($p \leq 0.05$) in the 3.0 mg/kg/day group (↑9%). Since dose-dependency was not observed in any of these findings, they are all considered to be incidental to treatment. No other differences ($p \leq 0.05$) were observed.

2. Gross pathology: No effects of treatment were observed on gross pathology. In the males, abscess of the epididymides was observed in one 12.5 mg/kg/day animal and in one control. Additionally, focus was observed in the liver of one 3.0 mg/kg/day male, and pelvic dilation of

the kidney was noted in one 1.0 mg/kg/day male. Since these findings occurred in single animals and dose-dependency was not observed, these findings are considered to be incidental to treatment. No other lesions were observed.

3. Microscopic pathology: Microscopic pathology was not performed.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The NOAEL under the conditions of this study was 3.0 mg/kg/day for brain and serum cholinesterase. The NOAEL for erythrocyte cholinesterase was 1.0 mg/kg/day.

B. REVIEWER COMMENTS: No effects of treatment were observed on clinical signs, mortality, food consumption, water consumption, hematology, clinical chemistry, organ weight, or gross pathology. In treated males, there was minimal decrease in absolute body weights and it was considered equivocal.

Decreases ($p \leq 0.05$-0.01) in cholinesterase activity were observed. Inhibition of either erythrocyte or serum ChE activity was seen on day 2 of examination period. On Day 8 in the 12.5 mg/kg/day females only, erythrocyte cholinesterase activity was decreased ($p \leq 0.01$) by 34%.

On Day 29 in the 12.5 mg/kg/day males, decreases ($p \leq 0.05$-0.01) were observed in serum cholinesterase (↓23%), erythrocyte cholinesterase (↓70%), and brain cholinesterase (↓65%) activities. On Day 29 in the females, decreases ($p \leq 0.01$) were observed at 12.5 mg/kg/day in serum cholinesterase (↓38%), at ≥3.0 mg/kg/day in erythrocyte cholinesterase (↓26-70%), and at 12.5 mg/kg/day in brain cholinesterase (↓70%) activities.

The LOAEL for serum and brain cholinesterase inhibition was 12.5 mg/kg/day. The NOAEL was 3.0 mg/kg/day.

The LOAEL for erythrocyte cholinesterase inhibition was 3.0 mg/kg/day. The NOAEL was 1.0 mg/kg/day.

This 28-day repeated dose oral toxicity study is classified as **acceptable/guideline** and satisfies the Guideline requirements (OPPTS 870.3050; OECD 407) for a 28-day oral toxicity study in rats.

C. DEFICIENCIES: No deficiencies were noted.

DATA FOR ENTRY INTO ISIS

Subchronic (28 day) Oral Study - rodents (870.3050)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
035001	46288001	subchronic	rat	28 days	oral	diet	1.0-12.5	0, 1.0, 3.0, 12.5	3.0	12.5	BW, BWG, FE	